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Solvent-Free Sonochemical Synthesis and Antifungal Activity of 1-Alkyl-3-Methylimidazolium Bromide [RMIM]Br Ionic Liquids

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In view of the continuous threat of opportunistic fungal infections to human health and the emerging importance of ionic liquids in therapeutic applications, we report the efficient one-pot synthesis of a series of 1-alkyl-3-methylimidazolium bromide [RMIM]Br ionic liquids through an ultrasound-assisted reaction of 1-methylimidazole and alkyl bromides (RBr) under solvent-free conditions. High product yields were obtained for all syntheses (>95%) under mild conditions (2-5 hours at 20-40 °C). The success of the synthetic method was confirmed through ^{1}H-NMR, ^{13}C-NMR and FT-IR spectroscopy. All products exhibited activity against the fungus *C. albicans* with clotrimazole and water as positive and negative controls, respectively. At a concentration of 1%, [OMIM]Br IL exhibited an antimycotic activity with an index of 1.5 which is comparable to that of 1% clotrimazole having an antimicrobial index of 1.3, signifying the potential of the product as a fungal growth inhibitor. Structure-Activity Relationship (SAR) studies showed that an increase in the alkyl chain length corresponds to an increase in the antifungal activity of the ionic liquids.

Keywords: Solvent-free; Sonochemical; Ionic liquid; Antifungal; *C. albicans*.

INTRODUCTION

Fungal infections pose a continuous threat to human health and life. In recent years, the frequency and severity of fungal infections has increased, predominantly on immunologically compromised individuals.[1] Pathogens such as *Candida albicans*, *Cryptococcus neoformans*, *Pneumocystis carinii* and *Aspergillus fumigatus* are the causes of considerable morbidity and mortality among patients with impaired immunity.[1] Although there are several antifungal agents that are readily available, the widespread use of these agents has resulted in the development of resistance of pathogenic microorganisms to these drugs.[2] As a result, morbidity and mortality has increased which then expresses the need to develop more promising and effective antifungal agents for clinical use.

Many effective antifungal agents used nowadays contain an azole moiety. Azole-based drugs inhibit the enzyme cytochrome p450 lanosterol 14 α-demethylase by binding in the active site of the enzyme, which then block ergosterol synthesis and ultimately, hinder fungal growth.[2] On this basis, it is suggested that imidazolium-based ionic liquids may also exhibit antifungal characteristics.

Ionic liquids (ILs), in general, consist of a salt where one or both ions are large, and the cation has a low degree of symmetry. These properties tend to reduce the lattice energy of the crystalline form of the salt, hence lowering the melting point which usually occurs below 100 °C.[4] Due to the attractive features of ionic liquids (negligible vapor pressure, high thermal stability, and considerable conductivity)[3-4] and the possibility of tuning these properties to suit various syntheses, extractions, catalyses, and applications, it is an undeniable fact that, as 'designer solvents', ionic liquids would continue to attract huge attention in the field of research.

Initially, the focus of research for ionic liquids was on their role as alternative green solvents in organic synthesis or reactions.[4-6] However after the synthesis of an ionic liquid derived from the antifungal compound miconazole,[7] researchers have begun to shift to the concept of "task specific ionic liquids". Since it is possible to introduce structural modifications either in the cation, anion or even the substituent on the ions of an ionic liquid, this allowed researchers to tailor these compounds to a specific application. For instance, 1-alkylquinolinium bromide ionic liquids have been studied for their antimicrobial and antibiofilm activities.[8] Myles and others, on the other hand, focused on synthesizing imidazolium derived ionic liquids with low toxicity and antimicrobial property while retaining their efficiency as Brønsted acidic catalysts.[9] Researchers also applied an ionic liquid (didecyldimethylammo-

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nium nitrate [DDA]NO_3) to linen fabric as to improve its resistance against fungal molds.[10]

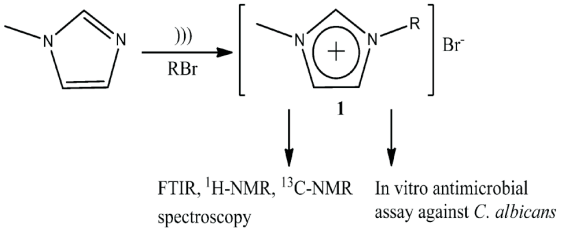
In the present work, we report the efficient sonochemical synthesis of a series of 1-alkyl-3-methylimidazolium bromide [RMIM]Br ionic liquids as confirmed by FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. This study also screened the ionic liquids for antifungal activity, and the effect of the length of the alkyl chain substituent on the bioactivity was evaluated.

RESULTS AND DISCUSSION

Synthesis of 1-Alkyl-3-Methylimidazolium Bromide [RMIM]Br Ionic Liquids

The sonochemical preparation of 1-alkyl-3-methylimidazolium bromide ILs follows the general scheme presented in Scheme 1. The synthesized products are yellowish in color. It is known that with colored starting materials such as 1-methylimidazole, a discoloration is difficult to avoid. Fortunately, these colored impurities are reported to be below the detection limit of NMR spectroscopy.[11] It was also observed that the apparent viscosity of the ILs increases as the length of the alkyl substituent on the cation increases. The observed viscosity can be attributed to the increase of the strength of van der Waals forces between molecules, causing a slower molecular motion hence, an increased viscosity. As shown in Table 1, the product yields ranged from 95-98%, rendering that the one-pot solvent-free sonochemical synthetic protocol is an ideal approach for a green and efficient generation of ionic liquids.

Scheme 1 General Schematic Diagram of the Study



Characterization of the Ionic Liquids

All synthesized products, including two other previously prepared ILs ([C_{12}MIM]Br and [C_{16}MIM]Br), were characterized based on NMR and FT-IR spectroscopic data to confirm success of synthesis. Differing only on the length of the alkyl group attached to the cation, a general spectral analysis for all products is provided.

The upfield ^{1}H-NMR signals (δ = 0.8-4.4 ppm) indicated the successful attachment of the alkyl group onto the tertiary N of methylimidazole, forming the quaternary ammonium salt. From the abovementioned chemical shift range, the one with the highest value is designated to the protons nearest the electronegative N atom, whereas the lowest value is assigned to the protons of the last carbon in the chain. On the other hand, the downfield peaks (δ = 7.2-10.6 ppm) are due to the protons attached to the sp^2 carbons of the imidazolium ring.

Table 1. Solvent-free Sonochemically-mediated Synthesis of ILs

Ionic Liquid	Reaction Time (min)	Yield (%)
[EMIM]Br	120	95
[BMIM]Br	180	96
[HMIM]Br	240	95
[OMIM]Br	300	98

The ^{13}C-NMR spectra for [RMIM]Br showed three downfield signals (δ = 121, 123, and 138 ppm) for the CH substituents on the imidazolium ring and two upfield peaks (δ = 14 and 37 ppm) which is assigned to the methyl (-CH_3) group of the alkyl chain and on the ring, respectively. Conversely, a set of peaks (δ = 22-50 ppm) is designated for the –CH_2 groups forming the alkyl chain. Thus, the number of signals corresponds to the number of carbons in the chain, excluding the methyl group at the end of the chain. Results showed 1, 3, 5, 7, 11, and 15 peaks for R = C_2, C_4, C_6, C_8, C_{12}, and C_{16}, respectively.

Functional group analyses of [RMIM]Br using FT-IR data confirmed the synthesis of the desired ILs. Prominent C=N stretch (1627-1632 cm^{-1}, m), C=C symmetric stretch (1566-1570 cm^{-1}, m), C-N stretch (1165-1176 cm^{-1}, s), and aromatic C-H stretch (3059-3144 cm^{-1}) peaks proved the presence of an imidazolium ring. Aliphatic C-H stretch signals were also observed at 2850-2986 cm^{-1} due to the alkyl chain group. The broad peak (3363-3537 cm^{-1}) can be attributed to the hydration of the ionic liquid sample.[3]

Evaluation of Antifungal Activity

The microbiological evaluation carried out on all products involved *in vitro* assays for antifungal activities. First, pure concentrations of the [RMIM]Br ILs were tested against *C. albicans* to verify their antimycotic properties.

As shown in Table 2, all synthesized ionic liquids exhibited high antifungal activities against the test microorganism. To determine a reasonable concentration for these potential antifungal agents, solutions having different concentrations of [OMIM]Br were prepared and tested for

Table 2. Screening Results of pure ILs Against *C. albicans*

Sample	Average Clearing Zone (mm)	Antimicrobial Index
A. [EMIM]Br IL	39	2.9
B. [BMIM] Br IL	33	2.3
C. [HMIM] Br IL	37	2.7
D. [OMIM] Br IL	46	3.6

their antifungal activity. For comparison, 200 µg of commercially-available Canesten® which has 1% clotrimazole (active ingredient) was dissolved and also subjected to the same assay as the positive control. Distilled water, on the other hand, served as the negative control.

From the data given in Table 3, it can be observed that an increase in concentration corresponds to a relative increase in the antimicrobial activity; however, the trend does not follow a strict linear relationship. Moreover, at a definite concentration (i.e., 1%), [OMIM]Br IL exhibited an antifungal property precise to that of the positive control (clotrimazole). Hence, it is plausible that [OMIM]Br can match the efficiency of clotrimazole as growth inhibitor of *C. albicans*.

Structure-Activity Relationship Studies

Structure-activity relationship (SAR) studies were also performed for the [RMIM]Br ILs as to determine the effect of a longer alkyl chain substituent on the antifungal activity against *C. albicans*. All six ionic liquids were diluted with distilled water to 1% concentration and subjected to bioassay.

Results of the SAR studies showed that the length of the alkyl chain attached to the cation affects the antifungal activity of [RMIM]Br ILs. Examination of the figure suggested that as the length of the carbon chain increases, the activity increases as well. However, the trend is nonlinear

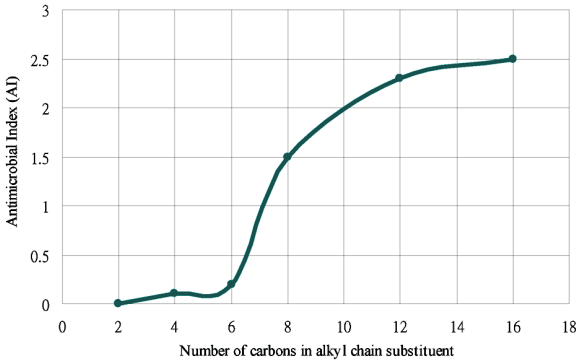


Fig. 1. Effect of the Length of Alkyl Chain Substituent on the Bioactivity.

Table 3. Assay Results of [OMIM]Br Standard Solutions

Sample	Average Clearing Zone (mm)	Antimicrobial Index
A. 1% [OMIM]Br	25	1.5
B. 5% [OMIM]Br	24	1.4
C. 10% [OMIM]Br	38	2.8
D. 25% [OMIM]Br	43	3.3
E. 50% [OMIM]Br	43	3.3
F. 100% [OMIM]Br	46	3.6
G. 1% clotrimazole	23	1.3
H. Water	-*	0.0

*No inhibition of growth of the test organism

Table 4. Screening Results of 1% [RMIM]Br IL Solutions

Sample	Average Clearing Zone (mm)	Antimicrobial Index
A. [EMIM]Br	-*	0.0
B. [BMIM]Br	11	0.1
C. [HMIM]Br	12	0.2
D. [OMIM]Br	25	1.5
E. [DDMIM]Br	33	2.3
F. [HDMIM]Br	35	2.5

*No inhibition of growth of the test organism

due to the abrupt increase in activity from [HMIM]Br to [OMIM]Br ILs. This sudden increase in activity can be attributed to the chemical structure of [OMIM]Br. The presence of a longer hydrocarbon chain or "tail" and a ionic imidazolium "head" impart greater amphipathy to [OMIM]Br IL. As a result, it can function as a surface-active agent or surfactant.[12-13] As a surfactant, it can effectively attack lipid structure, induce polar narcosis due to their interfacial properties, and ultimately cause membrane-bound protein disruption.[14] No inhibition activity was observed for [EMIM]Br IL which can be accounted to the regrowth of the hyphae after the cell's morphology is altered by the antifungal agent. A higher concentration would give complete inhibition and prevent hyphal regrowth.

EXPERIMENTAL

General: All chemicals (1-methylimidazole and alkyl halides) were purchased from Sigma-Aldrich and used without further purification except for ethyl acetate which was obtained from RCl Labscan and was distilled prior to use. The ionic liquids were obtained through irradiation at 40 kHz in an ultrasonic bath (Cole-Palmer), washed with ethyl acetate, and then dried under vacuum. All products were analyzed through ^{1}H-NMR and ^{13}C-NMR spectroscopy (Agilent Varian 500 MHz NMR spec-

trometer) and Fourier Transform Infrared (FT-IR) spectroscopy (Shimadzu IR Prestige21 spectrometer). Ionic liquid samples were diluted and tested for antifungal activity by the Microbiological Research and Services Laboratory of the University of the Philippines-Natural Sciences Research Institute.

Solventless Sonochemical Preparation and Characterization of the Ionic Liquids: The ionic liquids were synthesized adapting the method reported by Namboodiri and Varma.[15] A range of ILs containing 1-alkyl-3-methylimidazolium cations were synthesized by reacting 1-methylimidazole with alkyl bromides containing C_2 to C_8 hydrocarbon chain groups. In a 100-mL round-bottom flask, a reaction mixture of 60 mmol 1-methylimidazole and 120 mmol alkyl bromide was sonicated in an ultrasonic bath for 2-5 hours while attached to a reflux set-up until a viscous yellow liquid is obtained. The resulting liquid was cooled to room temperature, washed thrice with ethyl acetate in 20-mL portions to remove any unreacted starting materials, and then vacuum-dried for 3-4 hours at 80 °C to obtain the ionic liquid. All [RMIM]Br ionic liquids were characterized by ^{1}H-NMR, ^{13}C-NMR and FT-IR spectroscopy.

1a: 1-ethyl-3-methylimidazolium bromide [EMIM]Br: 95% yield; R_f value (DCM): 0.133; pH 7; IR (film) ν_{max}: 3537, 3140, 3097, 2986, 2874, 2067, 1632, 1570, 1454, 1338, 1169 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm) = 10.23 (s, 1H), 7.52 (dt, *J* = 7.3 Hz, 2H), 4.41–4.36 (m, 2H), 4.08 (s, 3H), 1.58–1.55 (m, 3H); ^{13}C-NMR (500 MHz, $CDCl_3$): δ(ppm) = 137.06, 123.56, 121.76, 45.28, 36.71, 15.65. **1b: 1-butyl-3-methylimidazolium bromide [BMIM]Br:** 96% yield; R_f value (DCM): 0.166; pH 7; IR (film) ν_{max}: 3476, 3140, 3059, 2954, 2931, 2870, 2067, 1627, 1566, 1462, 1165 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm) = 10.05 (s, 1H), 7.52 (dt, *J* = 7.4 Hz, 2H), 4.23 (t, *J* = 7.4 Hz, 2H), 4.01 (s, 3H), 1.82–1.76 (m, 2H), 1.27 (dq, *J* = 7.4 Hz, 2H), 0.84 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR (500 MHz, $CDCl_3$): δ(ppm) = 136.99, 123.73, 122.13, 49.70, 36.65, 32.05, 19.35, 13.37. **1c: 1-hexyl-3-methylimidazolium bromide [HMIM]Br:** 95% yield; R_f value (DCM): 0.233; pH 7; IR (film) ν_{max}: 3363, 3140, 3066, 2951, 2928, 2858, 2063, 1631, 1570, 1462, 1168 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm) = 10.18 (s, 1H), 7.51 (dt, *J* = 7.4 Hz, 2H), 4.26 (t, *J* = 7.3 Hz, 2H), 4.06 (s, 3H), 1.85 (td, *J* = 7.7 Hz, 2H), 1.30–1.21 (m, 6H), 0.83–0.78 (m, 3H); ^{13}C-NMR (500 MHz, $CDCl_3$): δ(ppm) = 137.19, 123.72, 121.99, 50.06, 36.70, 31.00, 30.18, 25.81, 22.30, 13.86. **1d: 1-octyl-3-methylimidazolium bromide [OMIM]Br:** 98% yield; R_f value (DCM): 0.333; pH 7; IR (film) ν_{max}: 3437, 3144, 3074, 2924, 2854, 2067, 1627, 1570, 1466, 1169 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm) = 10.17 (s, 1H), 7.49 (dt, *J* = 7.4 Hz, 2H), 4.28–4.25 (m, 2H), 4.07 (s, 3H), 1.88–1.82 (m, 2H), 1.30–1.16 (m, 10H), 0.80 (t, *J* = 6.9 Hz, 3H); ^{13}C-NMR (500 MHz, $CDCl_3$): δ(ppm) = 137.22, 123.70, 121.93, 50.08, 36.71, 31.58, 30.24, 28.93, 28.86, 26.17, 22.49, 13.98. **1e: 1-dodecyl-3-methylimidazolium bromide [DDMIM]Br:** IR (KBr) ν_{max}: 3068, 2924, 2850, 2060, 1570, 1466, 1168 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm) = 10.61 (s, 1H), 7.26–7.19 (m, 2H), 4.33–4.29 (m, 2H), 4.12 (s, 3H), 1.91 (dt, *J* = 7.5 Hz, 2H), 1.72 (s, 6H), 1.28–1.23 (m, 12H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (500 MHz, $CDCl_3$): δ(ppm) = 138.28, 122.88, 121.35, 50.30, 36.80, 31.88, 30.28, 29.57, 29.47, 29.34, 29.32, 28.96, 28.96, 26.25, 22.68, 14.13. **1f: 1-hexadecyl-3-methylimidazolium bromide [HDMIM]Br:** IR (KBr) ν_{max}: 3479, 3062, 2916, 2850, 2747, 2056, 1570, 1473, 1176 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm) = 10.46 (s, 1H), 7.38–7.26 (m, 2H), 4.32–4.29 (m, 2H), 4.12 (s, 3H), 1.95–1.82 (m, 6H), 1.29–1.22 (m, 22H), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (500 MHz, $CDCl_3$): δ(ppm) = 137.94, 123.15, 121.50, 50.25, 36.81, 31.91, 30.28, 29.69, 29.68, 29.67, 29.65, 29.63, 29.58, 29.49, 29.36, 29.35, 28.98, 26.25, 22.68, 14.13.

Antifungal Assay: The antifungal activities of the synthesized ILs and two other previously prepared ILs ([C_{12}MIM]Br and [C_{16}MIM]Br ILs) were determined by the Microbiological Research and Services Laboratory (MRSL) of the University of the Philippines Natural Sciences Research Institute (UP-NSRI). The test organism *Candida albicans* (UPCC 2168) used in the assay was obtained from the University of the Philippines Culture Collection (UPCC).

Microbial suspension was prepared from 24-hour old culture of the test organism. The suspending medium used was 0.1% peptone water. Pre-poured Glucose Yeast Peptone (GYP) Agar plates, about 3 mm thick, were inoculated with the microbial suspension by swabbing the agar surface. The cotton swab on an applicator stick was dipped into the microbial suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab was streaked over the entire agar surface. This procedure was repeated two more times, rotating the plate 60° each time to ensure even distribution of the inoculum. Three equidistant wells were made on the agar surface using a cork borer (10 mm diameter) and 200 μL of the sample was placed in each hole. A single well was done on the control plate. The plates were incubated at room temperature and observed after 24 hours. The clearing zone was measured in millimeters and the average diameter of the clearing zones was calculated. From these, the antimicrobial indices were determined. Screening results was compared with standard 1% clotrimazole and water as positive and negative controls, respectively.

CONCLUSIONS

In this study, we have successfully synthesized a se-

ries of 1-alkyl-3-methylimidazolium bromide [RMIM]Br ionic liquids through a simple, one-pot, solvent-free, sonochemically-mediated S_N2 reaction of 1-methylimidazole and alkyl bromides. This synthetic route offers a green and highly efficient route to ionic liquids as proven by high yields (>95%) even at benign reaction conditions. All products exhibited high antifungal activity against *Candida albicans* at pure concentrations. Canesten® (1% clotrimazole), on the other hand, gave an antimicrobial index (AI) of 1.3 which is comparable to that of [OMIM]Br IL which exhibited an AI of 1.5. The structure-activity relationship (SAR) studies showed that with the same anion (bromide), the longer the alkyl chain length of the cation, the higher the antifungal activity of the ionic liquid. However, the trend is nonlinear due to the capability of longer chain ILs to be surface-active. These preliminary results alongside with the observed properties of the ionic liquid should be taken into consideration for future researches. Further biological assays and tests such as cytotoxicological studies of these promising ionic liquids should be done to develop them as antifungal agents and determine their optimal concentration for clinical use.

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